Transfer of americium-241 from food and water to organs and tissues of the crucian carp*

T. Zotina, E. Trofimova, D. Dementyev and A. Bolsunovsky

Institute of Biophysics SB RAS, Akademgorodok, Krasnoyarsk, 660036, Russia, e-mail: t_zotina@ibp.ru

Abstract. The transfer of transuranium element americium-241 (^{241}Am) from food and water to organs and tissues of freshwater fish (*Carassius auratus gibelio*, crucian carp) has been investigated in laboratory experiments. The fishes were fed with homogenized biomass of submerged macrophytes labeled with ^{241}Am, through catheter. For the first two days after force-feeding, fishes excreted up to 80 % of ingested americium, for four days - 98 %. ^{241}Am was registered in internal organs and tissues that had no direct contact with americium (liver, bones, muscles) as early as two days after the feeding and after eight days, when the digestive tract was depurated. Among internal organs, the highest activity concentration was recorded in the liver (up to 427 Bq/kg). Activity concentration in bones (19-31 Bq/kg) was several times higher than in muscles, indicating the affinity of americium to bone tissue. Assimilation of ^{241}Am in organs and tissues of crucian carp from water occurred mainly via the digestive tract too.

1. INTRODUCTION

Americium-241 is a long-lived α-emitting artificial nuclide (half-life 432 y). The sources of ^{241}Am in aquatic ecosystems are discharges from nuclear fuel reprocessing plants and decay of ^{241}Pu. ^{241}Am is detected in sediments and aquatic weeds of the Yenisei River [1. 2] subjected to radioactive contamination from the Mining-and-Chemical Combine (ROSATOM) (Zheleznogorsk, 60 km downstream of the city of Krasnoyarsk).

^{241}Am, along with plutonium, dominates long term radiotoxicity. Hence it is important to know their biokinetics in aquatic food webs. Fish serve as direct diet connection of freshwater food webs with the human. ^{241}Am occurs in fish inhabiting freshwater environments contaminated with transuranium elements [5, 6, 10]. Freshwater fish can certainly accumulate radionuclides through both dietary and non-dietary pathways. Assimilation of ^{241}Am by marine fish has been thoroughly investigated experimentally [3, 4, 7, 8]. The experimental data on biokinetics of ^{241}Am in freshwater fish are scarce [11]. Uptakes of water-borne radionuclides can differ between freshwater and marine fish due to their

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physiological differences. This research was aimed to estimate and compare the assimilation of ^{241}Am by freshwater fish (crucian carp) from food and water.

2. MATERIAL AND METHODS

In our experiments we used freshwater herbivorous fish *Carassius auratus gibelio* (Bloch) (crucian carp). The age of the fish was 2 - 3 years, fresh mass of one individual - 22-49 g. Fishes caught in small ponds in the vicinity of Krasnoyarsk had been acclimated to laboratory experimental conditions (tap water, 20 ºC, aeration, photoperiod, dry food pellets) for 1-2 months. The fishes were kept starving for 5 days before experiments. During the experiments the fishes were not voluntarily fed.

Aquatic plant *Ceratophyllum demersum* L. labeled with ^{241}Am as described elsewhere [12] was used as a food source. The homogenate of ^{241}Am-labeled biomass was injected into the digestive tract of the fish, behind the pharyngeal tooth, with a silicone catheter mounted on a syringe. The injected ^{241}Am activity per fish averaged 9 or 23 Bq. After the force-feeding, fishes were incubated in isotope-free tap water for half an hour not to let the ingested ^{241}Am-labeled biomass get out. Afterwards, the fishes were kept in isotope-free tap water (1 l/fish) in triplicate per aquarium. The whole volume of water was changed daily; the aquariums were rinsed with 1 M nitric acid and the wash-off was added to the whole water sample.

To estimate the assimilation of water-borne americium, ^{241}Am was added to filtered (0.22 μm, GSWP, Millipore) tap water as nitric solution, pH was adjusted to 7 with NaOH, before the fishes were lodged in. Initial activities of americium in water were 300 and 140 Bq/l. After 5 days of incubation in americium-containing water (3 fishes per 3 l of water), fishes were incubated in isotope-free water for 30 minutes with water changed twice. At the end of the incubations, the fishes were quickly frozen. The 50-100-ml-samples of water were taken daily and the whole water volume was used for the measurement of americium activity at the end of incubation.

To measure americium activity, the samples of water were concentrated to 10 ml by evaporation, with addition of nitric acid. Fish bodies were dissected on aluminum foil. Organs and tissues were digested in the mixture of hydrogen peroxide (30 %) and nitric acid and concentrated to 10 ml by evaporation. Fresh mass of organs and tissues was (g): gills - 1.4-3.7, heads - 3.4-8.0, bones – 1.1-2.5, fins – 1.0-2.6, muscles – 8.3-17.0, skin and scales – 2.9-7.3, liver – 2.0-4.3, gonads – 0.4-1.3, sum of other organs (kidney, air-bladder, heart, blood) – 0.4-1.1, digestive tract – 0.4-1.2. The content of the digestive tract, if any, was carefully forced out and washed off from gloves with water. The mass was negligible and was not measured. The loss of total fresh mass was 9-18 % per individual. Samples from several fishes were combined to get statistically significant detectable activity (1 $\sigma < 10$ %).

Activity of ^{241}Am in samples was measured on a Wallac 1480 Wizard 3” gamma-counter (PerkenElmer, Finland). Activity concentration was calculated on a fresh mass basis. Assimilation of ^{241}Am was calculated as a ratio of ingested activity (Bq) to total activity in a fish body, excluding the digestive tract, and expressed as %.

3. RESULTS AND DISCUSSION

3.1. Assimilation of americium from food

Four days after force-feeding, fishes had been excreting greenish fecal pellets that consisted of incompletely digested biomass of plants. As we noticed before, crucian carps voluntarily grazing on *C.demersum* digested the plant biomass poorly too. For the first two days after force-feeding, fishes had excreted up to 80 % of ingested americium, for four days - 98 %. After eight days, no significant activity of americium was registered in water in one experiment, and just trace amounts of activity were registered in the other.

The activity of americium in organs and tissues of crucian carps was estimated at days two and eight after force-feeding (Table 1). At day two, the digestive tract of the crucian carp had not been completely depurated yet, and the highest activity of americium (82 %) was recorded in the content of the digestive tract (Table 1). Americium activity was, however, recorded in liver, bones and muscles (Table 1).

On day eight after force-feeding, the activity of americium in the digestive tract dropped to 12-34 %, but activity concentration was still rather high as compared to other organs and tissues (Table 1). The highest activity of americium was recorded in the liver (68-82 %) (Table 1). Hence, activity concentration of americium in the liver increased between days two and eight. Liver was also mentioned as a trap for americium in marine fish [4, 8] and mammals [9]. Bones and muscles assimilated up to 5-7 % of americium (Table 1). Activity concentration of americium in bones of crucian carp was 2.5-6.9 times higher than in muscles at day eight after feeding. A relatively high activity concentration of ^{241}Am in bones was also reported for marine fish [4, 8].

When the highest activity of americium (23 Bq/fish) was ingested by crucian carp, the activity in internal organs increased, and significant concentrations of americium were also registered in gonads (milts), kidney, etc. (Table 1).

Table 1. Activity of ^{241}Am in organs and tissues of crucian carp at days 2 and 8 after force-feeding with ^{241}Am-labeled food. Ingested activity of ^{241}Am was 9 Bq/fish and 23 Bq/fish. Mean values±sd.

Ingested activity of ^{241}Am, Bq/fish	Activity concentration, Bq/kg (Percent of total activity in the body, %)			Activity in organ as per cent of total assimilated activity, %		
	9	9	23	9	9	23
Day after force-feeding	Day 2	Day 8	Day 8	Day 2	Day 8	Day 8
Number of fishes	3	3	5	3	3	5
Organs and tissues						
Gills	16.5±3.8 (0.7)	6.6±1.6 (1.6)	33.5±2.6 (3.0)	9.8	2.4	3.6
Heads	13.1±1.8 (1.4)	2.1±0.7 (1.2)	23.7±1.5 (4.7)	18.8	1.8	5.8
Bones	6.5±4.7 (0.2)	19.2±2.7 (3.1)	30.5±4.7 (2.3)	3.0	4.6	2.8
Fins	<mda	6.9±2.4	35.8±3.1	0.0	1.8	3.0

		(1.2)	(2.4)			
Muscles	4.9±0.7 (1.3)	2.8±0.4 (3.5)	12.3±1.3 (5.7)	17.3	5.2	7.0
Skin and scales	3.1±1.8 (0.3)	2.6±0.8 (1.2)	24.3±1.6 (4.1)	3.8	1.8	5.0
Liver	44±3 (3.6)	155±8 (54.7)	427±26 (55.6)	47.3	82.3	68.1
Gonads	<mda	<mda	208±20 (6.1)**	0	0	7.0
Other organs*	<mda	<mda	127±9 (3.8)	0	0	4.7
Digestive tract	7417±46 (92.4)	458±21 (33.6)	337±25 (12.4)	Not considered		
Whole fish without digestive tract	11±1 (7.6)	20±2 (66.4)	70±5 (87.6)	100	100	100
Whole fish	142±3 (100)	29±3 (100)	78±6 (100)			

* Other organs: kidney, air-bladder, heart, blood; ** milts.

Assimilation of dietary ^{241}Am in fish bodies was estimated without taking into account the activity in the digestive tract. At day two after ingestion of ^{241}Am-labeled food 1 % of ingested ^{241}Am activity was assimilated in the whole body, at day eight – 3.8 %. The assimilation of americium in marine fish was estimated by Carvalho et al [4] as 0.1-1.7 % and by Mathews et al. [8] as 6-15 %. Assimilation of ^{241}Am in tissues of cuttlefish differed considerably in juvenile and adult individuals [3]. Excretion rate also varied with age. The assimilation of americium by marine fish was relatively low compared to ^{137}Cs assimilation (74 %) [8]. Bustamante et al [3] also reported essential differences in assimilation and excretion of ^{241}Am and ^{137}Cs for cuttlefish.

3.2. Assimilation of americium from water

Freshwater fishes do not need to ingest surrounding water permanently to provide osmotic regulation as marine fishes do. Freshwater fish can take in surrounding water to compensate for the excretion losses. Hence, we assumed that uptake of americium from water mainly occurred via gills and cover tissues. To check this hypothesis, we incubated crucian carp in water containing americium. To diminish the possibility of excretion losses we kept fish starved during the incubation experiments. After five days of incubation, considerable activity of americium was accumulated in fish bodies (Table 2). Among organs having direct contact with water-borne americium, the highest activity was registered in gills. That was to be expected because most intensive ion exchange with water occurs via gills, which have relatively large surface. The highest concentration factor of americium uptake from water in gills was reported for rainbow trout [11]. But in our experiments, the highest activity of americium (61-88 %) was registered in the digestive tract (Table 2). This is indicative of intake of surrounding water by crucian carp in spite of starvation. Further assimilation of americium evidently occurred via absorption in the digestive tract, similarly to diet intake. Americium was recorded in liver,

muscles, bones, gonads (Table 2). Activity concentration of water-borne americium assimilated in the body of crucian carp was 33-48 Bq/kg. Internal organs and tissues of crucian carp can be ranked based on accumulated activity concentration of americium from water as follows: digestive tract> gills> liver> bones=muscles (Table 2).

Based on the described experiments, we can suggest that in natural freshwater environments contaminated with americium the uptake of americium by crucian carp occurs mainly via the digestive tract, even when the radionuclide occurs in the water-borne form. Similar results were reported for marine fish [7]. During the anabiotic period of the year, which lasts in Siberia from October to May, americium can stay in the digestive tract of omnivorous or herbivorous fishes, which have long digestive tracts, for a long time, causing chronic exposure of internal organs to α-particles. Retention of americium in liver, gonads, bones etc. makes these organs targets for radiological effects too.

The retention of ingested americium in fish bodies indicates the possibility of the diet transfer of this actinide from aquatic ecosystems to inland ones. The retention of ^{241}Am in muscles of crucian carp makes the freshwater fish a real source of the actinide transfer to local population.

4. CONCLUSION

^{241}Am once ingested by crucian carp with labeled herbal food is rather quickly transferred from the digestive tract to internal organs and is retained in tissues and organs after the depuration of the digestive tract. Among internal organs, the highest activity concentration of americium was recorded in the liver (up to 427 Bq/kg). Activity concentration in bones (19-31 Bq/kg) was several times higher than in muscles, indicating the affinity of americium to bone tissue. Total assimilation of ^{241}Am in crucian carp was quite low (up to 4 %). Assimilation of ^{241}Am in organs and tissues of crucian carp from water mainly occurs via the digestive tract too. The retention of ^{241}Am in the digestive tract and other internal organs and tissues of crucian carp makes them a target for ionizing radiation (mainly α-particles). Long-duration seasonal anabiosis of herbivorous fish with long digestive tracts (similar to crucian carp) may cause chronic dose exposure. Assimilation of americium in muscles indicates the probability of the further transfer of americium to local population.

Table 2. Activity of water-borne ^{241}Am in organs and tissues of crucian carp after 5 days of exposure in water containing ^{241}Am. Initial activity concentration of ^{241}Am in water was 300±15 Bq/l and 140±10 Bq/l. Three and six fishes were used in the first and second experiments, respectively. Mean values±sd.

Organs and tissues	Activity concentration, Bq/kg (Portion in total activity in one fish, %)		Activity in organ / Total assimilated activity, %	
Initial activity of ^{241}Am in water, Bq/l	300±15	140±10	300±15	140±10
Gills	202±12 (3.5)	244±13 (22.4)	29.0	57.6
Heads	17.5±1.9 (0.7)	27.2±1.6 (5.3)	6.1	13.7

Bones	16.8±2.5 (0.2)	<mda	1.3	0.0
Fins	24.5±2.3 (0.3)	26.7±2.5 (1.7)	2.4	4.3
Muscles	11.6±0.7 (1.2)	1.5±0.3 (0.6)	10.1	1.6
Skin and scales	143±8 (4.4)	10±1 (1.8)	37.2	4.6
Liver	77±4 (1.3)	67±4 (7.1)	11.1	18.2
Gonads	42±4 (0.3)	<mda	2.8	0
Other organs	<mda	<mda	0	0
Digestive tract	2951±157 (88.1)	2494±135 (61.2)	Not considered	
Whole fish without digestive tract	48±3 (11.9)	33±2 (38.8)	100	100
Whole fish	399±22 (100)	84±5 (100)	-	-

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